

the collimated beam is directed against the objective lens, to shift the position of the optical trap, and (b) maintain the position of the collimated beam substantially fixed in the plane of the objective lens, so that the beam fills the lens at any angle and the light intensity of the trap is substantially independent of position.

The apparatus also includes an optical system for viewing the region of the chamber in which the optical trap can be moved. For detecting molecular fluorescence events, the optical system may include a laser illumination light for illuminating the manipulation region of the chamber with pulsed, high-energy coherent light.

Also disclosed is a method for preparing a polymer filament for microscopic examination in an extended condition. One end of the filament is coupled to a particle in the size range of about 10 nm to 10 μ m, preferably in the 0.1 to 1 μ m range, and the particle and filament are suspended in a fluid film in a chamber. With the other end of the filament anchored to the chamber, the particle is captured in an optical trap produced by directing a beam of divergent, coherent light through a collimating lens and directing the resulting collimated beam through a high-numerical aperture objective lens, as described above.

In one preferred embodiment, the trapping force of the optical beam is adjusted to a selected level, and the filament is stretched to a position at which the particle can just escape from the trap. The particle is then recaptured, returned to this position, and attached to the chamber, to place the filament under a selected stretching force.

In another preferred embodiment, the filament is fixed in its condition by fusing the particle to the chamber, using the heat of the optical trap to melt the particle at a selected filament-extended position.

The invention also includes a method of nucleic acid sample preparation, for examining the filament in an extended condition. In one embodiment, the filament is coupled at each end to a particle bead, such as by a phosphoamidate linkage. One of the particles is captured with the trapping beam in the optical trap and anchored to the chamber by optical welding, fusing the particle with the surface of the view chamber. The other particle is then captured in the trap and moved to place the filament in an extended condition. The stretching force applied to the filament in extension may be calibrated, to achieve a desired degree of filament stretching, and therefore a known relationship between observed linear distance along the filament and number of filament basepairs.

The extended nucleic acid filament may be examined in real time by fluorescence microscopy, for mapping or localizing the binding sites of sequence-specific fluorescence probes or enzymes, for measuring the kinetics of enzyme or ribosomal attachment to or movement along the filament, or for observing filament splicing events, such as are promoted by topoisomerase or recombination enzymes. The location of a fluorescently labeled binding molecule can be determined with a precision of between about 30-100 basepairs.

Alternatively, the extended DNA may be examined at high resolution (near basepair resolution) by nanometer-scale probe microscopy, such as force-filed microscopy.

These and other objects and features of the invention will be more fully understood when the following de-

tailed description is read in conjunction with the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic view of an optical trap apparatus constructed according to the present invention;

FIG. 2 is a schematic view of a chamber in the apparatus, showing the manipulation region where particle trapping and manipulation occurs;

FIG. 3 shows the ray optics of a spherical dielectric particle trapped in an highly convergent optical beam;

FIGS. 4A-4C illustrate the gradient force at the optical trap under conditions where a collimated beam fills the objective focusing lens (4A), where the same beam is shifted off-center, to move the position of the trap (4C), and where a small-width beam which does not fill the lens is used (4C);

FIG. 5 is a ray optics diagram illustrating movement of the optical trap;

FIGS. 6A-6C illustrate the steps in extending a DNA filament, and fixing the filament in its extended condition, in accordance with one embodiment of the invention;

FIG. 7 shows a hypothetical plot of filament stretching force as a function of filament length;

FIGS. 8A and 8B illustrate the steps in extending a duplex DNA at a final known stretching force;

FIGS. 9B and 9C illustrates steps in preparing an extended nucleic acid filament on a substrate, for high resolution microscopy; and

FIGS. 10A and 10B illustrate the use of the method of the invention for restriction fragment mapping in a large genomic fragment.

DETAILED DESCRIPTION OF THE INVENTION

I. Particle Manipulation Apparatus

FIG. 1 is a schematic view of a single-beam optical trap apparatus 10 constructed according to the present invention. A modified fluorescence microscope 12 in the apparatus provides part of the optical train in a single-beam optical trap, and also provides optics for viewing a region of a chamber 14 where particle manipulation takes place, in accordance with the invention. The chamber is mounted on a conventional microscope stage 16 which allows positioning in the plane of the stage, and vertical positioning, conventionally.

Considering first the components of the optical trap in the apparatus, a movable light source 18 is designed to produce a movable beam 20 of divergent coherent light. Source 18 includes an adjustable-power laser 22 which outputs a coherent optical beam. The laser may be a visible-light laser, such as an argon ion laser (514 nm), a near infrared diode laser (e.g., 830 nm), or an infrared Nd YAG laser (1.06 μ m). The power requirements are in the range 1 mW to 1 W.

The laser output beam is directed to a moveable platform 24 in the light source by an optical fiber 26 coupled conventionally to the laser. The fiber end is mounted on platform 24, and directs a source beam through a lens system which consists of a microscope objective lens 34 and a diverging lens 36. The lens system functions to decrease the divergence of the light out of the fiber. Platform 24 conventionally includes a pair of micrometers (not shown) for movement in the X-Y plane.